

We claim:

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- ✓ 1. An electron donor system for transferring electrons to enzymes with redox properties, wherein the system comprises an inorganic, non-electrode-bound source of electrons and a mediator which is able to transfer electrons from the source of electrons to the enzyme.
  2. An electron donor system as claimed in claim 1, wherein the enzyme is a cytochrome P450-containing enzyme.
  3. An electron donor system as claimed in claim 2, wherein the enzyme is a monooxygenase (E.C. 1.14.--).
  4. An electron donor system as claimed in any of the preceding claims, wherein the mediator has a standard normal potential in the region of less than about -0.4 V.
  5. An electron donor system as claimed in any of the preceding claims, wherein the mediator is selected from cobalt(III) sepulchrates, methylviologen, neutral red, riboflavin, ruthenium triacetate, FMN and FAD.
  6. An electron donor system as claimed in any of the preceding claims, wherein the source of electrons is a metal with a lower standard normal potential than the mediator.
  7. An electron donor system as claimed in claim 6, wherein the source of electrons is metallic zinc.
  8. An electron donor system as claimed in any of the preceding claims, selected from the systems:
    - Zn/cobalt(III) sepulchrates and
    - Zn/neutral red.
  9. A method for the enzymatic transfer of oxygen to a hydrocarbon-containing hydrogen donor molecule, which comprises incubating the hydrogen donor molecule in a reaction medium comprising the oxygen-transferring enzyme and an electron donor system as claimed in any of claims 1 to 8 in the presence of oxygen under reaction conditions.
  10. A method as claimed in claim 9, wherein the hydrogen donor molecule is selected from compounds of the formula

in which

5 R is an alkyl radical with 8 or more carbon atoms, and

X is a polar group capable of forming hydrogen bonds, preferably a carboxyl, amide, nitrile, sulfate, sulfone, amine or hydroxyl group.

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Sub Q3 11. A method for the enzymatic production of terminally or subterminally (position  $\omega$ -1 to  $\omega$ -4) hydroxylated fatty acids, which comprises

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a) converting a hydroxylatable fatty acid or fatty acid derivative in the presence of an electron donor system as claimed in any of claims 1 to 8 using a cytochrome P450 monooxygenase and oxygen; and

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b) isolating the hydroxylated product(s).

12. A method as claimed in claim 11, wherein the  $\omega$ -hydroxylatable fatty acid derivative is selected from terminally saturated, branched or unbranched fatty acids with more than 10 carbon atoms, in particular C<sub>12</sub> - C<sub>30</sub> fatty acids.

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Sub H 13. A method as claimed in any of claims 9 to 12, wherein the enzyme is a cytochrome P450 monooxygenase selected from:

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a) the wild-type enzyme which can be isolated from *Bacillus megaterium* (DSM 32T); or

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b) a mutant, which can be obtained by amino acid substitution in at least one of positions 26, 47, 72, 74, 87, 188 and 354, of the wild-type enzyme (SEQ ID NO: 35).

14. A method as claimed in claim 13, wherein a single mutant selected from F87A, F87V, L188K, V26T, R47F, S27G, A74G and M354T is employed.

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15. A method as claimed in claim 13, wherein the mutant has in position 87 the mutation F87A or F87V and at least one other of the following mutations: L188K, A74G, R47F and V26T.

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Sub Q5 16. A method as claimed in any of claims 11 to 15, wherein the electron donor system is zinc/Co(III) sepulchrate.

17. A method as claimed in any of claims 11 to 16, wherein at least stage a) is carried out in the presence of chloride ions.
- 5 18. A method as claimed in any of claims 11 to 17, wherein at least stage a) is carried out in the presence of a hydrogen peroxide-cleaving enzyme.
- 10 19. A bioreactor for use for producing  $\omega$ -hydroxylated fatty acids, which comprises immobilized monooxygenase and an electron donor system as claimed in any of claims 1 to 8 in a liquid reaction medium.
- 15 20. A detection method for fatty acid monooxygenases, which comprises
- 20 a) incubating an analyte suspected of having enzymic activity with an  $\omega$ -hydroxylatable fatty acid or fatty acid derivative which has a terminal chromophore or fluorophore which can be eliminated, in the presence of an electron donor system as claimed in any of claims 1 to 8; and
- 25 b) determining the elimination of the chromophore or fluorophore qualitatively or quantitatively.
- 30 21. A method as claimed in claim 20, wherein the conversion is carried out in the presence of a hydrogen peroxide-cleaving enzyme and, where appropriate, in the presence of chloride ions.
- 35 22. A test kit comprising an electron donor system as claimed in any of claims 1 to 8.

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